

WHAT IS CLAIMED IS:

1. A proteorhodopsin mutant having improved optical characteristics, said mutant comprising a mutation in a conserved residue of a wild-type proteorhodopsin variant,
5 wherein said proteorhodopsin mutant has lower pK_{rh} or less difference in maximum absorption wavelength between a basic and an acidic form, in comparison with the wild-type proteorhodopsin variant.
2. The proteorhodopsin mutant according to Claim 1, wherein said conserved residue is
10 a conserved histidine residue.
3. The proteorhodopsin mutant according to Claim 1, wherein said conserved residue is a conserved arginine residue.
- 15 4. The proteorhodopsin mutant according to Claim 2 or 3, wherein said wild-type proteorhodopsin variant is a naturally occurring proteorhodopsin variant of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127,
20 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, or 161; or other proteorhodopsin variants sharing at least 90% amino acid identity thereof.
- 25 5. The proteorhodopsin mutant according to Claim 2, wherein said conserved histidine residue is at amino acid position 77 of SEQ ID NO: 1 or position 75 of SEQ ID NO: 2.
- 30 6. The proteorhodopsin mutant according to Claim 2, wherein said proteorhodopsin mutant has a lower pK_{rh} in comparison with the wild-type proteorhodopsin variant.
7. The proteorhodopsin mutant according to Claim 2, wherein said conserved histidine residue is mutated to an amino acid capable of forming a hydrogen bond.

8. The proteorhodopsin mutant according to Claim 7, wherein said amino acid capable of forming a H-bond is asparagine, glutamine, lysine, arginine, tryptophan, serine, threonine, tyrosine, aspartic acid, or glutamic acid.
- 5 9. The proteorhodopsin mutant according to Claim 8, wherein said amino acid capable of forming an H-bond is asparagine, glutamine, lysine, tryptophan, aspartic acid, or glutamic acid.
- 10 10. The proteorhodopsin mutant according to Claim 3, wherein said conserved arginine residue is at amino acid position 96 of SEQ ID NO: 1 or position 94 of SEQ ID NO: 2.
- 15 11. The proteorhodopsin mutant according to Claim 3, wherein said proteorhodopsin mutant has less difference in maximum absorption wavelengths between a basic and an acidic form, in comparison with the proteorhodopsin variant.
12. The proteorhodopsin mutant according to Claim 10, wherein said conserved arginine residue is mutated to alanine, glutamic acid or glutamine.
- 20 13. An isolated nucleic acid sequence encoding the proteorhodopsin mutant according to Claim 1.
14. A proteorhodopsin mutant comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 163, 165, 167, 169, 171, 173, 175, 177, 179, 181
25 and 183.
15. An isolated nucleic acid sequence selected from the group consisting of SEQ ID NOs: 164, 166, 168, 170, 172, 174, 176, 178, 180, 182 and 184.
- 30 16. A method for preparing a proteorhodopsin mutant having improved optical characteristics comprising the steps of:
(a) identifying a conserved amino acid residue of a wild-type proteorhodopsin variant,

- (b) mutagenizing the conserved amino acid residue, and obtaining proteorhodopsin mutants,
- (c) determining the optical characteristics of the proteorhodopsin mutants, and
- (d) selecting the proteorhodopsin mutant having improved optical characteristics.
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17. The method according to Claim 16, wherein said conserved amino acid residue is histidine.
- 10 18. The method according to Claim 16, wherein said conserved amino acid residue is arginine.
- 15 19. The method according to Claim 16, wherein said conserved amino acid residue is mutagenized by site-directed mutagenesis.
20. The method according to Claim 16, wherein said improved optical characteristics are lower pK_{rh} or less difference in maximum absorption wavelength between a basic and an acidic form, in comparison with the wild-type proteorhodopsin variant.
- 20 21. A method of storing and retrieving optical data, comprising the steps of:
- (a) providing a film comprising a matrix having the proteorhodopsin mutant immobilized within,
- (b) exposing the film to light of a wavelength that is absorbed by the proteorhodopsin mutant at a resting state in a predetermined pattern,
- 25 (c) converting selective portions of the film to an excited state and storing optical data therein,
- (d) exposing the film of step (c) to light of a wavelength that is absorbed by the proteorhodopsin mutant at either a resting state or an excited state, and
- (e) detecting the stored optical data by an optical recording device.
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22. A light-driven energy generator comprising: (a) the proteorhodopsin mutant according to Claim 1, (b) a cell membrane, (c) a source of all-trans-retinal, and (d) a light source, wherein the proteorhodopsin mutant integrates within the cell

membrane to produce an integrated proteorhodopsin mutant, and the integrated proteorhodopsin mutant binds covalently to all-trans-retinal to produce a light absorbing pigment.

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